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Detection and Control of Bacterial Biofilms

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Abstract— *A biofilm is a clump of bacteria living in a self-produced matrix of extracellular polymeric substances (EPS) linked to a biotic or abiotic surface, indicating that biofilms can exist on a variety of biotic and abiotic surfaces. Abiotic surfaces include floors, walls, drains, equipment, and food-contact surfaces, as well as biotic surfaces like meat, the oral cavity, the intestine, the urogenital tract, and the skin. Humans are a good source of biotic microenvironments for biofilm and bacterial growth, which leads to infectious diseases in most cases. The optimum biotic environment for bacteria to thrive requires a supply of nutrients, humidity, and the right temperature. Biofilms originate on inert surfaces or dead tissue, and they're frequent on medical devices and dead tissue fragments, but they can also form on living tissues. Biofilms' tolerance to harsh environments provides a favorable habitat for microbial populations, allowing for a more efficient flow of chemicals and information amongst microorganisms. As a result, biofilm resistance is a self-protective strategy for microbial development. Bacterial biofilms are detectable by direct and indirect methods and they could be controlled. Bacterial biofilm is a major cause of antimicrobial-resistant bacteria's development and spread, causing severe infections and increased mortality rates.*

I. BACKGROUND TO THE STUDY

Biofilms are complex bacterial communities, which are embedded in a self-produced extracellular matrix (ECM). These biofilms play a crucial role in various fields, including medicine, food, and industry, where they can lead to the development of persistent infections, reduced efficiency in industrial processes, and contamination of food products. Therefore, the detection and control of bacterial biofilms are of utmost importance. This review provides an overview of the latest research on the detection and control of bacterial biofilms.

Detection of bacterial biofilms can be challenging, as they are often hidden and difficult to visualize. However, various techniques have been developed to detect bacterial biofilms, including microscopy, staining, and molecular methods. Microscopy techniques, such as confocal laser scanning microscopy (CLSM), can provide high-resolution images of the biofilm structure, and can also be used to detect the presence of specific bacterial species within the biofilm (Sandeep et al., 2018). Staining techniques, such as crystal violet staining, can also be used to visualize biofilms, but they are less specific than microscopy techniques (Muthuirulandi et al., 2020). Molecular methods, such as polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH), can be used to detect specific genes or bacterial species within the biofilm (Loo et al., 2020).

The control of bacterial biofilms can be challenging, as they are resistant to conventional antimicrobial treatments. Therefore, alternative strategies have been developed to control bacterial biofilms. One such strategy is the use of

antimicrobial peptides (AMPs). AMPs are small peptides that can penetrate the biofilm matrix and disrupt bacterial cell membranes, leading to cell death (Kim et al., 2019). Another strategy is the use of quorum sensing (QS) inhibitors. QS is a communication mechanism used by bacteria to coordinate gene expression within the biofilm. QS inhibitors can interfere with this communication, preventing the biofilm from forming or reducing its virulence (Alharbi et al., 2020). Additionally, physical methods, such as ultrasonication and photodynamic therapy (PDT), have also been used to disrupt bacterial biofilms (Lebeaux et al., 2014).

The detection and control of bacterial biofilms are important for the prevention of infections, food contamination, and industrial inefficiencies. Various techniques have been developed for the detection of bacterial biofilms, including microscopy, staining, and molecular methods. Alternative strategies, such as the use of AMPs, QS inhibitors, and physical methods, have also been developed for the control of bacterial biofilms. These strategies offer promising avenues for the development of new antimicrobial treatments and the prevention of biofilm-related problems in various fields.

II. DETECTION OF BACTERIA FORMING BIOFILMS

Biofilms generated by bacteria have been detected using a variety of approaches (Boakye *et al.*, 2019).

2.1 Direct observation

Biofilm imaging optical technologies such as light microscopy, SEM, TEM, and CLSM can be used to investigate the complexity and dynamics of biofilms. These methods are used to visualize 3D structures and determine whether or not biofilm exists.

2.1.1 Light microscopy

Light microscopy is the simplest, cheapest, most convenient, and fastest method for quantitatively observing the morphology of microorganisms adhering to surfaces and semi-quantitatively estimating the amount of microorganism adherent on surface (exist, absent, abundant, rare, etc.). Bacteria species such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* have been spotted using a light microscope on acrylic sheets of polymethacrylate films, glass cover slips, and polystyrene petri dishes. To improve the visual clarity of microorganisms, dyes such as epifluorescence and fluorescent can be utilized. Making a smear and centrifuging a sample, respectively, allows researchers to examine the morphologies of sessile and planktonic microorganisms using a light microscope (Kirmusaoglu, 2019).

2.1.2 Transmission electron microscope (TEM)

Due to photons and electrons penetrating cells weakly, thin section of cell cut is stabilized and stained by particular chemicals with the treatment of osmic acid, permanganate, uranium, lanthanum, or lead salts. These stains have a lot of atomic weight. If the exterior structure of cells is being observed, it makes little difference whether the cell section is thin or thick. Water content of biofilm is eliminated by graded dehydration with alcohol. Followed by resin infiltration, then the sample is encased in a gelatin capsule and sent to polymerization. After, thin section taken is post stained with uranyl acetate and lead citrate. At the end of all these stages, the material is examined using TEM ((Kirmusaoglu, 2019).

2.1.3 Scanning electron microscope (SEM)

SEM, a high-resolution technique based on surface scattering and electron absorption, is used to view biofilms because it can detect crucial structural components such as the presence of biofilm matrix (Bossu *et al.*, 2020). SEM is comparable to TEM, in SEM processing, instead of infiltration with resin, embedment in gelatin capsule, and staining with lead citrate and uranyl acetate like in TEM processing, the stage after dehydration is drying and coating the sample with gold. The sample is dried and coated with gold palladium after being dehydrated with graded alcohol. After all of these stages have been completed, the sample is examined using a scanning electron microscope (SEM) (Kirmusaoglu, 2019). However, SEM is a costly technology, and quantifying the biofilm is difficult, especially when researchers are unable to deal with live samples.

2.1.4 Florescent tagging of biofilm

2.1.2 Confocal laser scanning microscopy (CLSM)

CLSM is the most widely utilized approach for studying the 3D morphology of biofilm and it is used to investigate biofilms grown on flow cells with clear surfaces. For confocal microscopy and related methods, biofilm must be fluorescent due to fluorescent molecules such as green fluorescent protein (GFP), a fluorescent protein expressed by biofilm producer microorganisms within biofilm. Deep penetration of excited energy is achieved by scanning laser light across the sample. A 3D digital image is created by the fluorescence of intrinsic fluorophores such as GFP or chlorophyll, or molecules signaled by foreign probes such as fluorescent-labeled antibodies detected by a photomultiplier. CLSM screens the tridimensional shape and physiology of biofilms using a mix of molecular probes and fluorescent proteins that are designed to target and

visualize biofilm components. The majority of fluorescent proteins and probes are made to label cellular organelles and structures (Cruz *et al.*, 2021).

2.1.3 Fluorescent in situ hybridization (FISH)

The probes of the fluorescent in situ hybridization (FISH) technology can be used to identify specific bacteria present in a heterogeneous biofilm ecosystem. FISH can also be used to study fluorescently labeled bacteria within biofilm. Labeled DNA probes hybridize to their complementary nucleic acid targets in FISH. Kirmusaoglu (2019) states that a probe must be constructed to designate only a single species' conserved region. Peptide nucleic acid fluorescence in situ hybridization (PNA FISH) is also used to investigate the structure and composition of biofilms because it allows the use of several fluorescent probe labels that are distinctive of a single microbe. The PNA FISH technique is very useful for CLSM monitoring of mixed biofilms (Cruz *et al.*, 2021).

2.1.5 Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a technique for examining intractable and complex macromolecular and entire cell systems within biofilms. The NMR signal produced by excitation of the nucleus sample with radio signals is recognized with sensitive radio waves, and the biofilm sample is placed in a magnetic field. Because NMR examination of solids needs a dedicated magic angle spinning machine and may not produce similarly well-resolved spectra, the biofilm sample should preferably be dissolved in a solvent. NMR's timescale is relatively long, making it unsuitable for viewing quick processes, as it only produces an averaged spectrum (Nazir *et al.*, 2019).

2.2 Indirect observation

2.2.1 Roll plate method

On the outside surface of cylindrical materials such as catheters and vascular grafts, the roll plate method is used to detect suspected microbial colonization with the potential to cause indwelling device-associated infection. Microorganisms colonizing the catheter's external surface are discovered using the roll plate approach, rather than microorganisms colonizing the catheter's intraluminal location. (Kirmusaoglu, 2019) Material is touched and rolled on the medium's surface.

2.2.2 Congo red agar (CRA) method

The Congo red agar (CRA) method is a qualitative assay for detecting biofilm producer microorganisms based on the color change of injected colonies on CRA media. Congo red of 0.8 g and 36 g of sucrose are added to 37 g/L Brain heart infusion (BHI) agar to make the CRA medium. The sample to be observed is inoculated on the agar plate and incubated. The morphology of colonies with distinct colors classified as either biofilm producers or not is observed after a 24-hour incubation period at 37 °C. Biofilm producers have black colonies with a dry crystalline consistency, whereas non-biofilm producers have pink colonies (Kirmusaoglu, 2019).

2.2.3 Tube method (TM)

Tube method (TM) is a qualitative assay for detecting biofilm producer microorganisms when visible film is present. Isolates are inoculated in polystyrene test tube which contained Tryptic soy broth (TSB) and incubated for 24 hours at 37 °C. Planktonic cells are discharged by rinsing twice with phosphate-buffered saline (PBS) and sessile isolates with biofilms developed on the walls of polystyrene test tubes are stained with safranine for 1 hour. The stain is then removed by rinsing the safranine-stained polystyrene test tube twice with PBS. Visible film lining the walls and the bottom of the tube after the test tube process was air dried will be observed and this suggests biofilm development (Kirmusaoglu, 2019). The tube approach has the benefit of allowing the formation of a large biofilm mass that may be harvested simply by scraping the tube. One use for the technique could be to quantify the effect of antibacterial agents on biofilms by counting colony forming units recovered from tubes before and after treatment with the agents of choice (Nazir *et al.*, 2019).

2.2.4 Micro titer plate assay

The micro titer plate assay is a quantitative approach that uses a microplate reader to detect biofilm production. Bacterial suspension is prepared in Mueller Hinton Broth (MHB) which is supplemented with 1% glucose and adjusted to 0.5 McFarland (1×10^8 CFU/mL). This bacterial solution is diluted 20 times (1/20) to yield 5×10^6 CFU/mL. Then, 180 μL of MHB supplemented with 1% glucose and 20 L of bacterial suspensions are injected into a 96 well flat-bottomed sterile polystyrene microplate to reach a final concentration of 5×10^5 CFU/mL (tenfold dilution (1/10)). At 37 °C, the microplates are incubated for 24 hours. After planktonic cells in wells of microplate are discharged by washing twice with phosphate-buffered saline (PBS) and the wells are dried at 60 °C for 1 hour, sessile isolates with biofilms developed on the walls of wells of microplate are stained with only 150 L of safranine for 15 minutes. After that, the safranine-stained microplate wells are rinsed twice with PBS to remove the safranine stain. The dye of biofilms that lined the walls of the microplate is resolubilized by 150 L of 95 percent ethanol or 33 percent glacial acetic acid or methanol after air drying the wells of the microplate. A microplate reader then measures the microplate spectrophotometrically at 570 nm. The experiments are carried out three times. The blank absorbance readings are used to determine whether or not isolates develop biofilms.

Biofilm producers are isolates with optical density values greater than the blank well (Kirmusaoglu, 2019).

2.2.4 Detection of biofilm-associated genes by polymerase chain reaction (PCR)

PCR techniques are employed not only to identify infections by amplifying species-specific nucleic acid sequences, but also to detect virulence factors by amplifying target virulence genes such as biofilm genes using gene specific primers, even in the presence of an uncultured pathogen. Biofilm-associated gene forward and reverse primers are used. PCR, such as qualitative real-time PCR, multiplex, and conventional PCR, is used to detect whether biofilm-associated gene is present or not in microorganisms. The PCR result is seen on an agarose gel containing a DNA intercalating dye such as ethidium bromide to confirm the presence of amplified gene. The amplicon is only recognized by fluorescence in qualitative real-time PCR employing a pair of particular hybridization probes tagged with fluorescent dye (Kirmusaoglu, 2019).

2.2.5 Tissue culture plate method

Organisms are injected in 10 mL of TSB with 1% glucose after extraction from fresh agar plates. At 37 °C, the broths are incubated for 24 hours. The cultures are then diluted at 1:100 in fresh media. A 200-L portion of the diluted cultures are placed in individual wells of sterile 96-well flat bottom polystyrene tissue culture treatment plates. The organisms in the control group are also incubated, diluted, and added to a tissue culture plate. Inoculated sterile broth serves as the negative control wells. At 37 °C, the plates are incubated for 24 hours. The contents within each well are carefully tapped out after incubation. A 0.2-mL quantity of PBS is used to wash the wells four times. This eliminates the floating microorganisms. The biofilm generated by bacteria adhering to the wells is preserved with 2 % sodium acetate and stained with crystal violet (0.1 percent). The excess stain is washed with deionized water, and the plates is set aside to dry. The optical density (OD) of stained adherent biofilm is measured at 570 nm using a micro ELISA autoreader. The experiment is carried out three times, in duplicate (Hassan *et al.*, 2011).

III. CONTROL/ PREVENTION OF BACTERIAL BIOFILM FORMATION

For the safety of both non-medical and medical regions, a variety of microbial biofilm control approaches involving limited water and nutrient supply, controlled temperature, and well-designed apparatus are necessary. Disinfection and washing of surfaces where bacteria cling are the most common methods for preventing biofilm formation. Acidic chemicals, caustic products, aldehyde-based biocides, hydrogen peroxide, chlorine, iodine, ozone, isothiazolinones, phenolics, peracetic acid, surfactants, and biguanidines are all commonly employed in disinfection procedures. To disinfect and eliminate biofilms, mechanical therapy might be combined with chemical treatments (Tasneem *et al.*, 2018). Another method for preventing biofilm formation is to utilize small molecule biofilm inhibitors. A biofilm inhibitor's antibiofilm characteristics are frequently used to passivate the surface of an implanted medical device or biomaterial. A variety of biofilm inhibitors can be used such as phenols, imidazoles, furanone, indole, bromopyrrole and so on (Verderosa *et al.*, 2019).

Three major strategies for controlling biofilm formation or targeting different stages of biofilm growth have been discovered. The first strategy is to prevent bacteria from adhering to the biofilm-forming surface, the second strategy is to impede biofilm formation during the maturation process and the third strategy is to interfere with the bacterial communication system, also known as the quorum sensing (QS) system, which coordinates biofilm formation and maturation in bacteria (Subhadra *et al.*, 2018). Table 1 summarizes various antibiofilm techniques and agents used.

Table 1: Various strategies for the control of biofilms.

Strategy	Methods/Agents	Examples
Inhibition of initial biofilm attachment	(i) Altering chemical properties of biomaterials	(i) Antibiotics, biocides, iron coatings
	(ii) Changing physical properties of biomaterials	(ii) Use of hydrophilic polymers, super hydrophobic coatings, hydrogel coatings, heparin coatings
Removal of biofilms	(i) Matrix degrading enzymes	(i) Polysaccharide-degrading enzymes (Dispersin B, Endolysins); Nucleases (Deoxyribonuclease I) and Proteases (Proteinase K, trypsin)

	(ii) Surfactants	(ii) Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), Tween 20 and Triton X-100, surfactin, rhamnolipids
	(iii) Free fatty acids, amino acids and nitric oxide donors	(iii) Cis-2-decenoic acid, D-amino acids, nitric oxide generators such as sodium nitroprusside (SNP), S-nitroso-L-glutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP)
Biofilm inhibition by quorum quenching	(i) Degradation of QS signals	(i) Lactonases, acylases and oxidoreductases
	(ii) Inhibition of signal synthesis	(ii) Use of analogues of AHL precursor S-adenosyl-methionine (SAM), S-adenosyl-homocysteine (SAH), sinefugin, 5-methylthioadenosine (MTA), butyryl-SAM; SAM biosynthesis inhibitor cycloleucine, AHL synthesis inhibitors such as nickel and cadmium
	(iii) Antagonizing signal molecules	(iii) AHL analogues (bergamottin, dihydroxybergamottin, cyclic sulfur compounds, phenolic compounds including baicalin hydrate and epigallocatechin); AI-2 analogues (ursolic acid and phenyl-DPD); AIP analogues (cyclic peptides such as cyclo (L-Phe-L-Pro) and cyclo(L-Tyr-L-Pro), RNAIII inhibiting peptide (RIP) and its homologues)
	(iv) Inhibition of signal transduction	(iv) Use of halogenated furanone or fimbrolide, cinnamaldehyde, virstatin
	(v) Inhibition of signal transport	(v) Use of copper or silver nanoparticles, Phe-Arg-β-naphthylamide (PAβN)

Source: Subhadra *et al*. (2018)

3.1 Plant-derived antimicrobial compounds

Many medicinal plants have long been used to heal a variety of ailments. Plant-derived chemicals are both safe and cost-effective, with no known negative effects. Monoterpenoids (such as borneol, camphor, carvacrol, eucalyptol, limonene, pinene, thujone), sesquiterpenoids (such as caryophyllene, humulene), and flavonoids (such as cinnamaldehyde and other phenolic acids) make up the majority of plant-based essential oils (Campana *et al.,* 2017). Some of these essential oils have antibacterial and antibiofilm effects (Goel *et al.,* 2021).

3.2 Enzymes

Because enzymes are biodegradable and have a minimal toxicity, they are considered green counter measures against biofilm formation. These characteristics make them an effective biofilm control technique. The generated biofilm is dispersed using enzymes. Examples include: Xylanase, alpha-amylase etc. Xylanase, a cell wall disintegrating enzyme, reduced biofilm development by 70% and dispersed the *Pseudomonas aeruginosa* PAO1 biofilm without impacting planktonic bacteria (Goel *et al.,* 2021).

3.3 Polysaccharides

Polysaccharides can be utilized to prevent the production of biofilms. Most anti-biofilm polysaccharides block biofilms throughout a broad spectrum, whereas others can disperse preformed biofilms. Antibiofilm polysaccharides could be a potential technique for the treatment and prevention of biofilm-related infections due to their non-biocidal mode of

action, biocompatibility, and biodegradability. Antibiofilm polysaccharides are thought to be useful as an adjuvant because they improve antibiotic activity when given combined (Kostakioti *et al.*, 2013).

3.4 Biosurfactants

Biosurfactants are natural chemicals that can change the hydrophobic properties of the bacterial surface. This changes the qualities of adhesion and binding to any given surface. Biosurfactants prevent biofilm development by altering cell adhesion ability through reduced cell surface hydrophobicity, membrane rupture, and inhibition of the electron transport chain which lowers cellular energy demands (Mishra *et al.*, 2020). *Pseudozyma aphidis* DSM 70725, which produces new biosurfactants, produces mannosyl erythritol lipids (MELs). MELs prevents *Staphylococcus aureus* biofilm development by inhibiting bacterial adherence to the surface (Goel *et al.*, 2021).

3.5 Nanoparticles (NP)

The use of nanoparticle coated medicines to dissolve biofilms could result in biofilm eradication. Multidrug-resistant and biofilm-associated illnesses can be treated with nanoparticles instead of antibiotics. The biofilm-NP interaction is a three-step process: (1) NP transport around the biofilm, (2) NP attachment to the biofilm EPS, and (3) NP penetration into the EPS and migration within the biofilm through diffusion, which may be influenced by biofilm pore sizes, charges, hydrophobicity, and the EPS chemical gradient. AuNPs (gold nanoparticles), NO NPs (nitrous oxide-releasing nanoparticles), and drug-delivery NPs with targeting ligands, for example, have the ability to improve closeness between individual biofilm cells within the EPS and the nanocarrier. Because of their versatility, biocompatibility, targeted/triggered release, and ability to integrate lipophilic and hydrophilic medicines, lipid and polymer NPs are gaining popularity (Ekundayo *et al.*, 2021; Shrestha *et al.*, 2022).

3.6 Antibiofilm agents

Antibiofilm agents are a group of substances that can prevent and eliminate the production of biofilms. Antibiofilm substances are mostly derived from natural sources, however chelating agents and synthetic compounds have also been discovered to have antibiofilm action. Plakunov *et al.* (2019) divided the agents into four categories based on their activities at different stages of biofilm development, as shown in Table 2 and Figure 1 below:

Table 2: Classes of antibiofilm agents and their functions.

Antibiofilm agent	Functions
Class I	penetrate the biofilm EPS and decrease the growth of cells
Class II	interfere with the adherence of bacteria and the formation of biofilm phenotype
Class III	controls both the growth of bacteria with biofilm phenotype as well as the EPS synthesis
Class IV	disperse the mature biofilms

Source: Shrestha *et al.* (2022)

3.6.1 Surface attachment inhibitors

Controlling surface attachment has the potential to inhibit the entire biofilm formation process. The suppression of adhesin and EPS molecules can also prevent biofilm development. Surfactants, which reduce the interfacial tension between two substances, are a popular option of antimicrobial agents for limiting bacterial attachment to surfaces. Surfactants are amphiphilic because they have both a hydrophilic and hydrophobic component, and they can be classified as non-ionic, anionic, cationic, or amphoteric. Tween 80 (Polysorbate 80) and Triton X-100 are two commonly used non-ionic, synthetically produced surfactants in laboratories. Microorganisms produce surface-active substances called biosurfactants, which are made up of structurally varied biomolecules (Nitschke *et al.*, 2007).

Quaternary ammonium compounds (QACs) are cationic surfactants that are employed as disinfectants in the food industry and in a variety of medical problems. QACs bind to microorganisms' negatively charged regions causing cell wall stress, lysis, cell death and promote protein denaturation which lowers food intake by affecting cell wall permeability. Several biosurfactants have antibacterial properties, and some even appear to inhibit infections from colonizing surfaces. Rhamnolipid an example of biosurfactant promotes biofilm dispersal in *P. aeruginosa*, *S. aureus*, *Salmonella enteritidis*, and *L. monocytogenes* (Shrestha *et al.*, 2022).

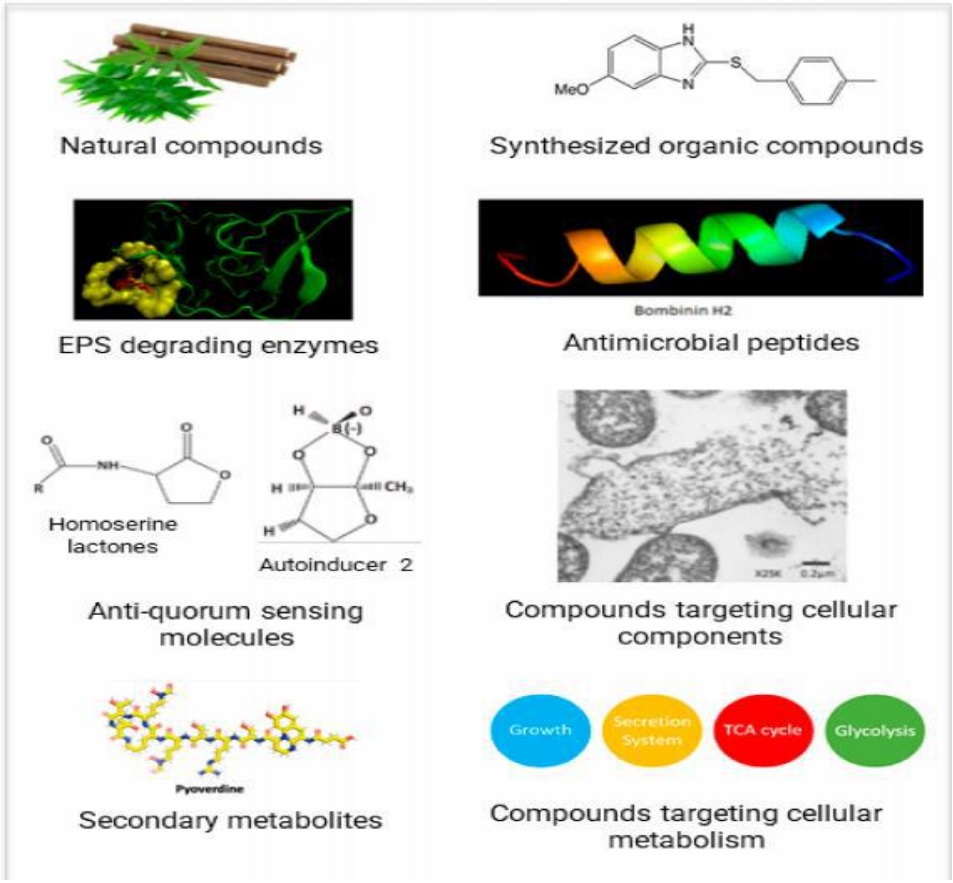

Fig.1: Antibiofilm agents

Source: Shrestha *et al.* (2022)

3.6.2 Compound inducing cell lysis

Biofilm formation may be inhibited by the breakdown of peptidoglycan, which affects the composition of teichoic acids and proteins on the cell wall and also releases signals that regulate genes involved in biofilm formation (Roy *et al.*, 2018). Transglycosylase and peptidoglycan hydrolases (endolysins) are enzymes that breach the cell wall and cause bacterial cell death. Chelating compounds like Ethylene diamine tetra acetic acid (EDTA) can damage cell walls, causing biofilms to break down by sequestering zinc, magnesium, iron, and calcium (Finnegan & Percival, 2015). As a result, bacteria can be combated early in the biofilm growth process by applying such chemicals (Shrestha *et al.*, 2022).

3.6.3 Anti-Quorum Sensing Molecules

Many natural and synthetic chemicals operate as anti-QS molecules, focusing on the QS signaling molecules, this is listed in Table 3. Ichangin and isolimonic acid are potent repressors of biofilm and the type III secretion system, as well as strong regulators of cell-to-cell signaling in bacteria. Cinnamaldehyde, another natural chemical, can decrease swimming motility and change biofilm structure and development especially in *Escherichia coli*. Hordenine, a strong phenylethylamine alkaloid derived from barley can reduce the production of the signaling molecule and impact biofilm development (Zhou *et al.*, 2018). At lower concentrations, plant polyphenols known as quercetin dramatically inhibit biofilm development and other virulence factors (Shrestha *et al.*, 2022).

Table 3: Natural compounds as anti-quorum sensing molecules in biofilm dispersal.

Compound/Molecule	Mode of Action	Effective Against
Garlic extracts	inhibits QS	*Pseudomonas aeruginosa*
Garlic extracts	inhibit LasR and LuxR	*Pseudomonas aeruginosa*
Querentin	decrease LasI/R, RhlI/R expressions	*Pseudomonas aeruginosa*

Isolimonic acid	cell-to-cell signaling	*Escherichia coli*
Isolimonic acid	reduce LuxR DNA binding	*Vibrio* spp.
Cinnamaldehyde	swimming motility	*Escherichia coli*
Hordenine	decrease in signaling molecule, inhibition of QS-related genes	*Pseudomonas aeruginosa*
Autoinducing peptide type I (AIP-I)	inhibit QS	*Staphylococcus aureus*
RNAIII-inhibiting peptide (RIP)	inhibit QS	*Staphylococcus aureus*

Source: Shrestha *et al.* (2022)

3.6.4 Synthetic Small Organic Molecules

The development of synthetic small organic compounds has created a new path for overcoming antibiotic tolerance and disrupting biofilms. Some imidazole and benzimidazole chemicals have the ability to both inhibit and disperse biofilms. By targeting eDNA, polysaccharide intercellular adhesion (PIA), and Protein A (SpA) expression (Shrestha *et al.*, 2016). The biofilm inhibitors indole-3-carboxaldehyde and 3-indolylacetonitrile chemicals reduce biofilm formation by inhibiting curli generation while leaving microbial growth unaffected. Biofilms are reported to be inhibited by brominated furanone derivatives in a variety of bacterial species.

3.6.5 Antimicrobial Peptides (AMP)

Antimicrobial Peptides (AMP) are cationic and hydrophobic residues that contain compounds that can interact with bacteria, fungi, protozoa, and some enveloped viruses. Some AMPs can suppress biofilm in a variety of pathogens at sub-minimal inhibitory concentrations (MICs), hence these peptides are known as antibiofilm peptides (ABPs). Cleavage of peptidoglycan, change of membrane permeabilization or membrane potential, neutralization or disassembly of lipopolysaccharides, inhibition of cell division and cell survival, modulation of adhesion molecule synthesis and function, and repression of the stringent response of bacteria are all antibiofilm effects of antimicrobial peptides (Andrea *et al.*, 2018; Roy *et al.*, 2018). Examples of AMPs are nisin, bovicin HC5, D-enantiomeric protease-resistant peptides, Peptide 1037 and so on. Peptide 1037 can inhibit biofilm formation by reducing swarming and swimming motilities, generating twitching motility, and suppressing numerous biofilm-related genes.

3.6.6 Compounds Targeting Metabolism

This agents or compounds inhibit biofilm formation by modifying it metabolism and affecting the bacterial biofilms genes. Examples are: tea tree oil which have antibacterial and antibiofilm effect against *Staphylococcus aureus*, and it can also modify its metabolism by changing the expression of genes involved in the pyrimidine, purine, glycine, serine, and threonine metabolism pathways, as well as the amino acid biosynthesis route. Exogenous amino acids, such as L-arginine, also inhibits biofilm development by suppressing the genes required for the creation of *Streptococcus mutans* biofilm EPS (Shrestha *et al.*, 2022).

3.6.7 EPS Degrading Enzymes for biofilm Dispersal

The use of EPS degrading enzymes such as amylase, Dispersin B (DspB), and DNase I to break down the EPS is a common antibiofilm approach which reduces biofilm development and degrades mature bacterial biofilms such as *Vibrio cholerae, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Sun *et al.*, 2013). EPS degrading enzymes have the potential to be employed as an antibacterial agent in biofilm dispersal strategies.

3.6.8 Phage Therapy

Bacteriophages, commonly known as phages, are bacterial viruses that are bacteria's natural enemies. Many bacteriophages also produce depolymerases, which destroy the EPS in biofilms, making them excellent for biofilm targeting (Mishra *et al.*, 2020). Phage therapy uses lytic phages, which do not enter a prophage form and so rarely carry or transfer virulence genes, while causing rapid bacterial cell destruction (Kostakioti *et al.*, 2013). Some phages have hydrolytic enzymes on their surface that allow them to infiltrate the biofilm matrix and infect bacteria within biofilms. Bacteriophages have a number of characteristics that make them sensitive to biofilms. Bacteriophages like Sb-1 can boost antibiotic activity against biofilm (Shrestha *et al.*, 2022).

One advantage of phage therapy over antibiotic therapy is that it is considerably more targeted. In contrast to antibiotics, which can kill both harmful and beneficial bacteria in the stomach, a phage adheres to one specific bacterial strain while leaving others intact. At the same time,

the specificity of phage therapy may be a disadvantage because matured and naturally generated biofilms may be resistant to phage therapy (Shrestha *et al*., 2022). However, there are still some drawbacks to using phages, such as the risk of bacterial resistance to phages, the possibility of unwanted horizontal gene transfers via lysogenic phages to share virulence-related genetic elements across the biofilm community, and phage immunogenicity, which can result in the human host producing neutralizing antibodies, which can lead to inflammatory side effects (Schulze *et al.*, 2021). Multiple bacteriophages can be mixed to generate a super phage mix, also known as a phage cocktail, to increase the action of phage against biofilms. Bacteriophages are extremely promising technologies for controlling or even eradicating bacterial biofilms (Luo *et al.,* 2021).

3.6.9 Photodynamic therapy

It makes use of photosensitizing compounds, which absorb light of a given wavelength and binds to target cellular components such as lipids, proteins, and nucleic acids. It generates reactive oxygen radicals, which in turn form hydrogen peroxide, hydroxyl radicals, and superoxide anion, killing or poisoning the target. For this type of mechanical elimination of biofilms using photodynamic treatment, the photosensitizer and light source used are critical. Methylene blue, toluidine blue, and toluidine blue O are some of the most commonly used photosensitizers (Sadekuzzaman *et al.*, 2015). Methylene blue has a wavelength of 664 nm, while toluidine blue has a wavelength of 638 nm, both of which are in the UV-visible range of 600-1000 nm. It has been discovered that the amount of time spent pre-radiating over the target has an effect on the microorganism's elimination (Srinivasan *et al.*, 2021).

3.6.10 Quorum quenching (QQ)

Quorum quenching (QQ) is the phenomena of the QS system being down-regulated or silenced. Suppression of QS signal molecule formation, signal sequestration, receptor antagonist, and inhibition of targets in the QS signal transduction pathway are all examples of QQ techniques (Srinivasan *et al.,* 2021). Chemistries, antibodies, and specialized enzymes can all be used to sequester signal molecules. For managing bacterial biofilm, peptide-based quorum sensing modulators are being actively explored, and this method looks to be more effective for gram-positive bacteria. Phytochemicals and plant by-products are two forms of anti-QS compounds that are very promising. Anti-QS agents are mechanistically sound, implying a novel class of biofilm-fighting compounds (Luo *et al.*, 2021).

Quorum quenching can be done at multiple levels utilizing different chemicals, such as preventing bacterial adhesion, inhibiting biofilm formation, or causing mature biofilms to disintegrate. Although quorum quenching does not kill germs, it does make them more susceptible to conventional treatments and can be used in conjunction with antibiotics (Schulze *et al.*, 2021). Anti-QS drugs can theoretically disrupt QS signaling and hinder biofilm formation because QS plays such a vital part in biofilm formation signaling. As a result, anti-QS drugs may be able to combat antibiotic resistance brought on by biofilm development (Luo *et al.*, 2021). Resistance to quorum sensing inhibitors (QSIs) can only emerge as a result of mutations that prevent QS-deficient bacteria from producing virulence factors; as a result, the bacteria become nonvirulent (Li *et al.*, 2020). Lactonase, acylase, oxidoreductase, and paraoxonase are all examples of quorum quenching enzymes found in bacteria.

The inactivation of acyl homoserine lactone molecules is the recognized mode of action of QQs (Sadekuzzaman *et al.,* 2015). Furanone, ajoene, naringin, musaceae, and curcumin are some of the natural QSIs that have been shown to suppress bacterial biofilm formation. Furthermore, the presence of a secondary messenger called c-di-GMP in high concentrations encourages bacteria to develop biofilms. As a result, blocking the c-di-GMP pathway could be a good technique to avoid biofilm formation (Muhammad *et al.*, 2020). Quorum quenchers, on the other hand, are usually species specific; thus, to eradicate mixed-species biofilms, a mixture of quenchers is required. In both Gram-negative and Gram-positive bacteria, ajoene, a sulfur-rich compound from garlic, reduces the expression of small regulatory RNAs (sRNAs) (Mishra *et al.*, 2020).

3.6.11 Electrochemical method

The electrochemical approach is one of the most interesting and promising strategies for preventing bacterial biofilm formation. The electrochemical technique, often known as the 'Bioelectric effect,' is the result of combining a lower dose of antibiotics with a mild electric field to disintegrate biofilm development or mature biofilm. The electric potential reduces the antibiotic dosage required to inactivate the biofilm and causes the biofilm organisms to die. The essential principle of the electrochemical method is that under direct current, electrostatic force enhances antimicrobial binding and transport towards the biofilm matrix, hence increasing biofilm detachment efficacy. The media undergoes hydrolysis as a result of the electric field, resulting in the release of charged ions and hyper oxygenation in response to heat stimuli (Srinivasan *et al.*, 2021).

Antibiotics usually have a difficult time penetrating the biofilm matrix. The antimicrobial agents cause the biocide ions to be released under the influence of the electrical field, which is ascribed to a change in biofilm permeability. The

biofilm is inactivated as a result of the entry of biocide ions into the biofilm matrix. Even at low concentrations, it kills bacterial cells by electrophoresis and electro-osmosis. Electrospray is another novel way for eliminating biofilms using the electrochemical technology. A sterile polymer surface devoid of biofilm is obtained by dispersing fluids from a high energy potential (Srinivasan *et al.*, 2021).

IV. BACTERIAL BIOFILM CONTROL IN DRINKING WATER DISTRIBUTION SYSTEM (DWDS)

The following are some strategies for limiting the formation of bacterial biofilms in the drinking water distribution system:

4.1 Pretreatment

This is accomplished by lowering the amount of organic matter entering the distribution system. Microbial growth is controlled by limiting the nutrients required for growth through more appropriate DW treatments (sedimentation, filtration, UV disinfection, ozone, and peroxide), i.e. the formation of biologically stable DW. Microorganisms require a 100: 10: 1 C: N: P (carbon, nitrogen, and phosphorous) ratio, with carbon being the growth-limiting nutrient. As a result, limiting the carbon content reduces the likelihood of microbial growth. Biofilm generation on pipe surfaces can be controlled or delayed using an aqueous suspension of silver nanoparticles as a pre-treatment in water systems prior to the main treatment units, such as membrane filtration (Simoes & Simoes, 2013).

4.2 Material selection

This ensures that the piping and fittings are built from materials that are chemically and biologically stable. The type and stability of the material used in DWDS can have a significant impact on biofilm proliferation. Biofilms develop at varying rates and have varied microbial community structures in different types of pipes. Iron pipes sustain 10 to 45 times greater growth than plastic pipes, and it is also more reactive to disinfectants and quenching their antibacterial properties. As a result, the type of material can have an impact on biofilm disinfectant efficiency. Biofilms grown on copper, Polyvinyl chloride (PVC), and cement lined ductile iron were inactivated with far less free chlorine or mono-chloramine than biofilms grown on unlined iron surfaces (Simoes & Simoes, 2013).

4.3 Hydrodynamics

This refers to the prevention of water stagnation and silt collection in distribution systems. Pipes with long water residence durations and dead-ends are linked to high organic material sedimentation zones and, as a result, profuse biofilm growth. Periods of non-flow or the storage of water in residential pipes or tanks are linked to high bacterial populations. To reduce sediment build-up in DWDS, operation measures such as pre-treatment optimization, minimizing particles in DW entering the network, the application of sufficiently high flow velocities that may result in a self-cleaning network, and regular flushing under specified conditions should be considered (Simoes & Simoes, 2013).

4.4 Chemical disinfection and alternate techniques

This refers to keeping a suitable level of disinfectant throughout the distribution system. Chemical disinfection, primarily with chlorine, and an increase in its residual content throughout the network are the key strategies for controlling biofilm growth in DWDS. Water disinfection is a technique for killing or inactivating microorganisms that have survived the treatment process and ensuring microbiologically safe water through the DWDS. This is accomplished by using excessive disinfectants, notably chlorine, to maintain a disinfectant concentration during water distribution, so preventing microbiological formation in pipelines and tanks (Simoes & Simoes, 2013).

A handful of pathogenic germs, however, are resistant to chlorine. Chloramines (less efficient than free chlorine and produces the same Disinfection byproducts (DBPs) as chlorine but in lesser levels), Ozonation, and UV radiation (electromagnetic energy in the range 250-265 nm) are examples of disinfectants that can be employed in DWDS. Physical (UV light) and chemical (chlorine and chlorine dioxide) treatments in combination are more effective in removing DW biofilms than either treatment alone (Simoes & Simoes, 2013).

4.5 Bacterial biofilm control in food industries

Biofilm generation is controlled in the food industry using a variety of physical means and chemical compounds, including within pipelines and on work surfaces. The prevention of biofilm formation in the industry is a critical step in achieving the goal of a safe and high-quality product. However, it is impossible to completely avoid or eliminate biofilm growth on food and in the food processing environment. In the food sector, bacterial biofilms are treated as follows:

4.5.1 Chemical Treatments

As a biofilm treatment, a variety of concentration- and time-dependent chemical sanitizers can be used. The goal is to lower microbial populations to safe levels for humans, which is known as sanitization. Food processing equipment must be sanitized in order to prevent cross contamination between batches of food. Chlorine-based sanitizers are most commonly employed in the food industry; however, some microorganisms have developed resistance to chlorine

treatments. Aqueous Chlorine dioxide (ClO_2) is the most extensively used sanitizer in the food sector (Nam *et al.*, 2014). In the food industry, quaternary ammonium compounds (such as Metaquats) are frequently employed as sanitizers, including for biofilm eradication. The bacterial cell membrane is disrupted by these positively charged water soluble chemicals, resulting in bacterial lysis (Jennings *et al*., 2016). The clearance of biofilms generated by these resistant bacteria could be improved with a multi-faceted approach involving a mix of therapies. Other less prevalent sanitizers, such as salicylate-based polyanhydride esters can be used (Galie *et al.*, 2018).

4.5.2 Enzymatic Disruption

Because enzymes are biodegradable and have a minimal toxicity, they are considered green countermeasures against biofilm formation. They are commonly employed in detergents for food industry applications because of these characteristics, which make them an effective tool for biofilm reduction. Pectin methylesterase, for example, is an enzyme which can inhibit biofilm formation in bioreactors. The food industry needs this activity because it can be used as a pre-treatment for various devices and equipment (Galie *et al.,* 2018). Other enzyme activities, such as amylases, cellulases, lyases, glycosidases (such as dispersin B), and DNAses, are often utilized in the food industry as part of industrial detergents to remove biofilms (Galie *et al.,* 2018).

4.5.3 Steel Coatings

Nanoparticles have antibacterial qualities and can be used in a variety of industrial settings by coating n surfaces of equipment colonized by bacterial biofilm. In industry, silver nanoparticles and metal oxide nanoparticles are more widely used. Iron oxide (Fe_3O_4), titanium oxide (TiO_2), zinc oxide (ZnO), copper oxide (CuO), and magnesium oxide (MgO) are some of the examples of nanoparticles (Galie *et al.,* 2018).

4.5.4 Biosurfactants

Biosurfactant can be used in food industry surfaces to reduce the adhesion of germs like Methicillin-resistant *Staphylococcus aureus* (50 percent adhesion inhibition at 8.3 g/mL) and other microorganisms. These chemicals operate on the surface of the relevant target microorganism, lowering surface tension and modifying binding capability. Chelating cations are chemicals that attach themselves into the membranes of microbial cells. This impact changes the permeability of the cell membrane, eventually destroying it and resulting in cell enlargement and death (Galie *et al.*, 2018).

4.5.5 Bacteriocins

Bacteriocins are used in the food industry to inhibit biofilm growth on various surfaces. These antimicrobial compounds can also extend a product's expiry date, protect it from changes during refrigeration, reduce food spoiling, limit the transmission of foodborne pathogens, lower chemical preservative concentrations, and reduce the number of temperature treatments. Nisin is the only FDA-approved bacteriocin in the food industry, and when used as a spray on food-processing surfaces, it can prevent *Listeria monocytogenes* adherence and biofilm development (Galie *et al.,* 2018).

4.5.6 Essential oils

Monoterpenoids (such as borneol, camphor, carvacrol, eucalyptol, limonene, pinene, thujone), sesquiterpenoids (such as caryophyllene, humulene), and flavonoids (such as cinnamaldehyde and other phenolic acids) make up the majority of plant-based essential oils (Campana *et al.*, 2017). According to Desai *et al.* (2012), oregano and thyme oils were likewise found to be highly effective at eradicating *Listeria monocytogenes* biofilms on polystyrene and stainless steel surfaces. Carvacrol is also efficient against biofilms formed by *Listeria monocytogenes* and *Staphylococcus aureus* (Galie *et al.,* 2018).

Other methods for inhibiting or preventing bacterial biofilms include high hydrostatic pressure, non-thermal plasma, quorum sensing inhibition, bacteriophages (phage therapy), and photocatalysis (Galie *et al.,* 2018). The control methods and action mechanism is shown in Table 4 below:

Table 4: Biofilm control methods for their use in the food industry

Methodology	Examples	Mechanism of action
Chemical treatments	Sanitizers (NaOCl, peracetic acid, NaOH, H_2O_2)	Cell structures oxidation
Enzymatic disruption	Cellulase Proteases	Extracellular matrix disruption

	Glycosidases	
	DNAses	
Steel coatings	Nanoparticles (Ag^{2+}, Fe_3O_4, TiO_2, ZnO, CuO, MgO)	Alteration of bacterial membrane
	Repelling surfaces (monolayers, hydrogels, modified topography)	Inhibition of bacterial binding
	Functionalized surfaces (with lisozyme or nisin)	Bactericidal
Biosurfactants	Lichenysin	Inhibition of bacterial adhesion
	Surfactin	
Bacteriophages	P100	Cell lysis
Bacteriocins	Nisin	Cell membrane alteration
QS inhibition	Binding of inhibitors to QS receptors (lactic acid)	Down regulation of adhesion and virulence mechanisms
	Enzymatic degradation of QS signals (paroxonases)	
	sRNA post-transcriptional control	
	Inhibition of QS signals biosynthesis	
	Furanones	Motility inhibition
Essential oils	Citral	QS inhibition, motility inhibition
	Carvacrol	Bactericidal
High hydrostatic pressure	H_2O	Bactericidal (also endospores)
Non-thermal plasma	UV plus O_2, N_2, O_3, H_2O and He	Bactericidal
Photocatalysis		Bactericidal

Source: Galie *et al.* (2018)

4.6 Bacterial biofilm control in healthcare facilities

Biofilm formation on medical devices could be avoided by altering the surface qualities of the devices to prevent bacteria from attaching to them. To detach biofilms from tissues or reduce their effect, higher doses of antimicrobial medicines might be utilized.

4.6.1 Use of nanoparticles

Silver nanoparticles are frequently employed for managing biofilms for medical devices. Actually, the charged silver ions aid in the static attraction between the metal and the charged microbe, enhancing absorption and antibacterial activity through the membrane. Silver nanoparticle treatment slows DNA replication, ribosomal and other cellular protein expression, and interferes with microbial Electron transport chain (ETC). This approach has been confined to human tissues due to the potential toxicity of silver ions. The inclusion of chelators/chelating chemicals destabilizes the biofilm architecture. Calcium, magnesium, and iron are well-known for maintaining membrane integrity and, when combined with a tetrazolium EDTA chelator, for fighting biofilms in vitro or on explanted tube tips, as well as for treating catheter-related blood infections (Malhotra *et al.*, 2015).

4.6.2 By Altering the Chemical Properties of Biomaterials

Antibiotics, biocides, and ion coatings are popular chemical approaches for modifying the surface of biomedical devices to avoid biofilm formation. Antibiotic-coated catheters, such as minocycline and rifampin, have been found to reduce the incidence of *Staphylococcus aureus* biofilm-associated bloodstream infection in hospitals (Ramos *et al.*, 2011). Catheters impregnated with various antibiotics, such

as nitrofurazone, gentamicin, norfloxacin, and others, are also thought to play a role in reducing biofilm-associated urinary tract infections. Antibacterial agent coatings on medical equipment are often only effective for a short length of time due to the chemical's gradual leaching. Thus, utilizing long, flexible polymeric chains to immobilize antimicrobial chemicals on device surfaces has proven to be an efficient way to limit biofilm formation in the long run (Subhadra *et al.*, 2018).

4.6.3 Surfactants

Surfactants such as sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), Tween 20, and Triton X-100 aids biofilm dispersal and detachment. Surfactin, a cyclic lipopeptide generated by *Bacillus subtilis* has been shown to prevent biofilm formation and stimulate biofilm dispersal in *Salmonella typhimurium, Escherichia coli*, and *Proteus mirabilis* (Subhadra *et al.*, 2018). Many bacteria, including *Pseudomonas aeruginosa*, synthesize rhamnolipids, which promote biofilm dispersal in a variety of bacterial strains.

4.6.4 Anti-adhesion Coatings

For the eradication of biofilms on clinical surfaces, there are primarily four chemical cleaning procedures used. Detergent, hydrogen peroxide cleaning, bactericidal/bacteriostatic coatings, and anti-adhesion coatings are examples of these approaches. Anti-adhesion coatings may typically prevent biofilm formation at an early stage, which is preferable in therapeutic settings. Chemical composition and reactivity, hydrophilic/hydrophobic properties, surface textures, and surface charges are all factors to consider when designing an anti-adhesion coating surface. For example, Li *et al.* (2020) found that the modified polyurea antibiofouling coating has a hydrophobic property, that nanotitanium dioxide can generate reactive oxygen species to kill bacteria, and that the riblet surface textures formed by nanotitanium dioxide can improve the drag reduction effect and antibiofouling performance. Furthermore, such coatings have the ability to extend the interval between maintenance and demonstrate their commercial relevance in real-world applications (Li *et al.*, 2020).

Quorum quenching, the utilization of free fatty acids, amino acids, and nitric oxide donors, the use of matrix degrading enzymes, and so on are all examples of other control mechanisms (Subhadra *et al.*, 2018).

V. CONCLUSION AND RECOMMENDATION

Biofilms are surface-attached communities of bacteria held together by self-produced polymer matrixes made mostly of polysaccharides, secreted proteins, and nucleic acids [RNA and extracellular DNA (eDNA)], as well as other components such as water, lipids, extracellular enzymes, and metal ions which poses a severe threat to public health of individuals. The EPS in biofilm allows surface adhesion and serves as a barrier between biofilm cells and the environment which provides nutrients and protect them from desiccation, radiation and other environmental conditions. Biofilm is formed in a variety of ways by various organisms. Bacterial biofilm development takes place in a series of well-ordered steps from initial attachment to surfaces to maturation of the biofilm. Biofilm growth is influenced by a variety of biotic and abiotic variables such as oxygen requirement, pH, nutrients availability and so on. Biofilm-associated bacteria differ from their free-living planktonic type in a number of ways.

To establish a biofilm on any surface, such as implant materials, vessels, pipes, water bodies, food items, textile surfaces, ship hulls, power plants, and so on, a wet or hydrated area with some nutrients is the minimal condition. On the surface, biofilm production is a slow and laborious process that takes years to develop and mature. The chemical composition, surface area, and stability of the substratum colonized by the biofilm microbiota have significant implications for its structure and function, as well as distinguishing its communities within and between habitats, the amount and kind of cells in the biofilm, as well as the external physical environment, are all critical considerations. These parameters have an impact on biofilm production and responsiveness to environmental challenges such as antibiotic or chemical treatment.

Various procedures and approaches have been developed in order to get rid of dangerous biofilms, with the main focus on interfering with bacterial attachment and QS, as well as biofilm matrix degradation. Biofilm generation in various industrial equipment can be treated using a variety of standard mechanical and chemical procedures such as the use of biosurfactants, enzymes, nanoparticles on surfaces and so on. Novel strategies, such as the use of anti-adhesion agents to block a specific biofilm step without killing the bacteria, or the use of natural bacterially produced signals to promote bacterial dispersal are bioavailable treatment strategies for biofilm eradication.

The complexity of biofilm-mediated infections and their increased resistance to antibiotics make them difficult to control. The prevention of their surface colonization to restrict biofilm development is important, as this is the first step in the formation of biofilms. Future strategies to improve biofilm eradication may be developed to encourage the commercial intake of certain biofilm inhibitors like enzymes, AMP, AML, and QS inhibitors. However, in-

depth research is required for the clarification of the effect of these biofilm inhibitors during biofilm infection in the host while their applicability to humans should also be proven. It should be noted that biofilm inhibitors may not be responsible for antibiotic resistance; they hold a lot of promise in the future for treatment or management of biofilm-based infections.

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